A flow chart for array-based detection of gene expression

Hybridization oligo: 3' [=====] 5'
  D   EX   Zip   U

U: upstream universal priming site
Zip: unique sequence as a molecular "zip-code"
EX: gene-specific exon sequence
D: downstream universal priming site

Exon sequence

mRNA: Cap ———————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA

Exon sequence

Cap ———————————(A)n

3' 5'

Amplify signal by PCR:
(D primer is biotinylated)

B —————→
3' [=====] 5'
          ←—————

Hybridize and
detect:

B 3'

zip-code array
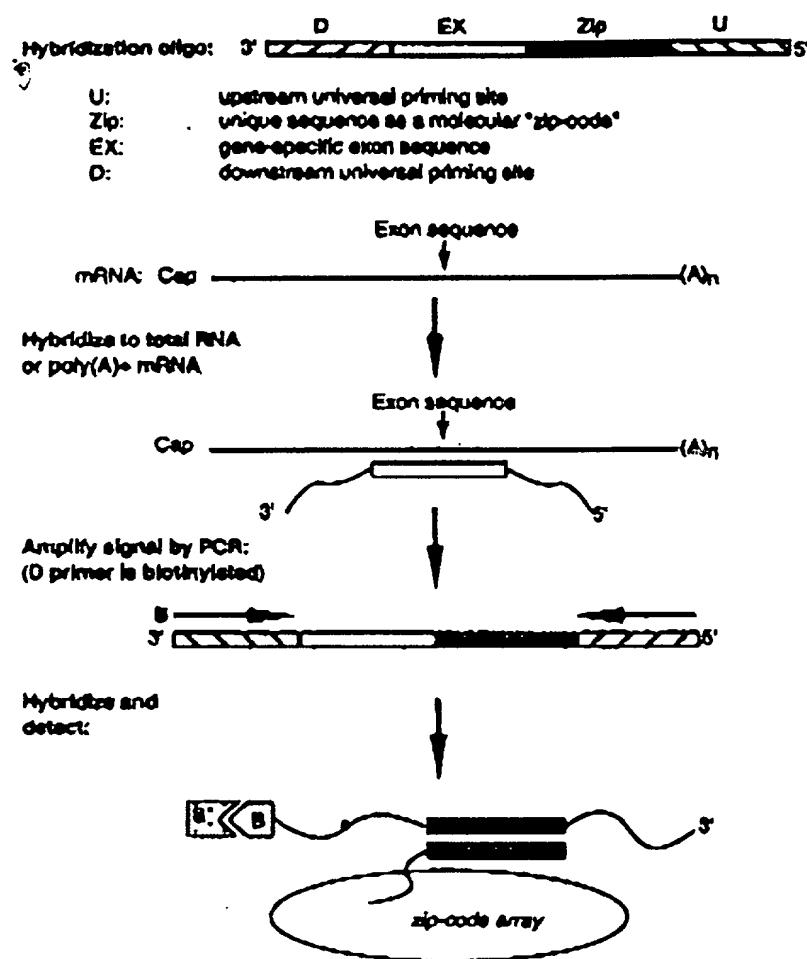
Figure 1

A flow chart for array-based detection of RNA alternative splicing

Hybridization oligo: 3' [D] [SJ] [Zip] [U] 5'

U: upstream universal priming site
Zip: unique sequence as a molecular "zip-code"
SJ: gene-specific splice junction
D: downstream universal priming site

Splice junction
↓
mRNA: Cap ————————————————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA

↓

Splice junction
↓
Cap ————————————————————(A)n
        3'   5'

Amplify signal by PCR:
(D primer is biotinylated)

↓

3' [━━━] [━━━] 5'

Hybridize and
detect:

↓

zip-code array
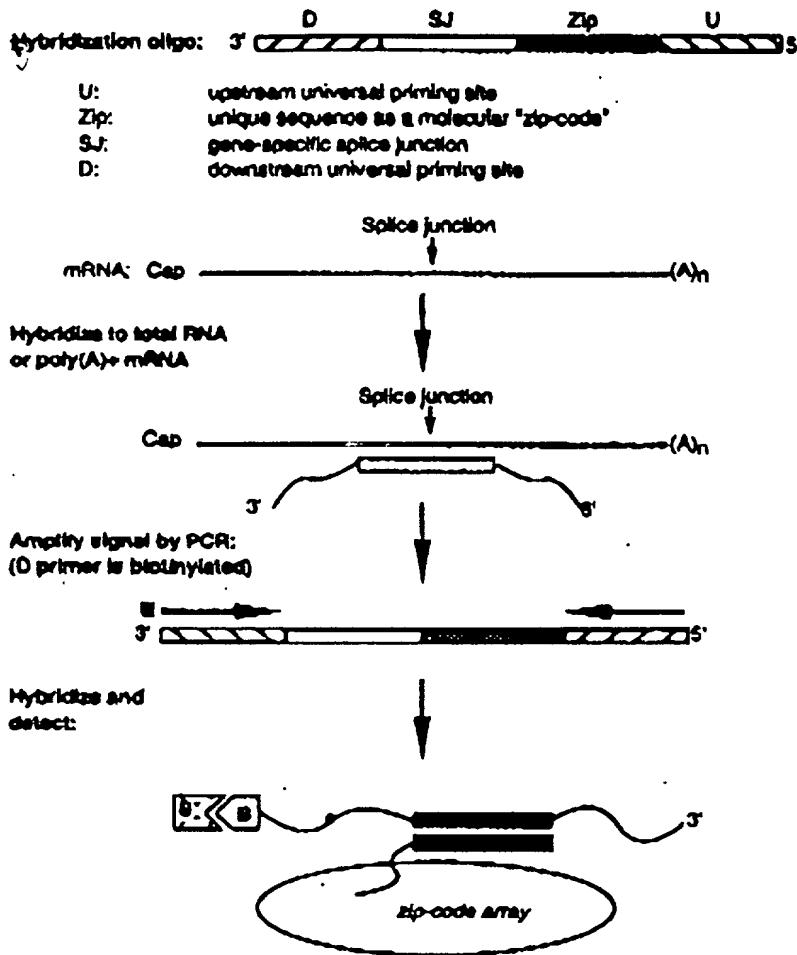
Figure 2

Genome-wide gene expression profiling using oligo-ligation strategy

EX1   Zip 1   U

Upstream oligo: 3' [_____] 5'

Downstream oligo: 5' [_____] 5'
                         D   Zip 2   EX2

U: upstream universal priming site
Zip 1:   unique sequence as a molecular "zip-code"
Zip 2:   a different zip-code
EX1:   gene-specific exon sequence
EX2:   gene-specific exon sequence
D: downstream universal priming site

Exon sequence

mRNA: Cap ——————————————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA:

mRNA: Cap ——————————————————(A)n

Ligate the oligos: 3'   5'

mRNA: Cap ——————————————————(A)n

Amplify Signal by PCR: 3'   5'
(D primer is biotinylated)

B   B
3' [_____] 5'

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

3'

zip-code array

Zip2

3'

Figure 3

Genome-wide RNA alternative splicing monitoring using oligo-ligation strategy

Upstream oligo:

SJ   Zip 1   U

Downstream oligo:

D   Zip 2   ES

U:	upstream universal priming site
Zip 1:	unique sequence as a molecular "zip-code"
Zip 2:	a different zip-code
SJ:	gene-specific splice junction
ES:	exonic sequence adjacent to the splice junction
D:	downstream universal priming site

splice junction

mRNA: Cap ———————————————(A)n

Hybridize to total RNA
or poly(A)+ mRNA:

splice junction

mRNA: Cap ———————————————(A)n

Ligate the oligos:

splice junction

mRNA: Cap ———————————————(A)n

Amplify Signal by PCR:
(D primer is biotinylated)

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Zip1

zip-code array

Zip2

Figure 4

Direct genotyping using a whole-genome oligo-ligation strategy
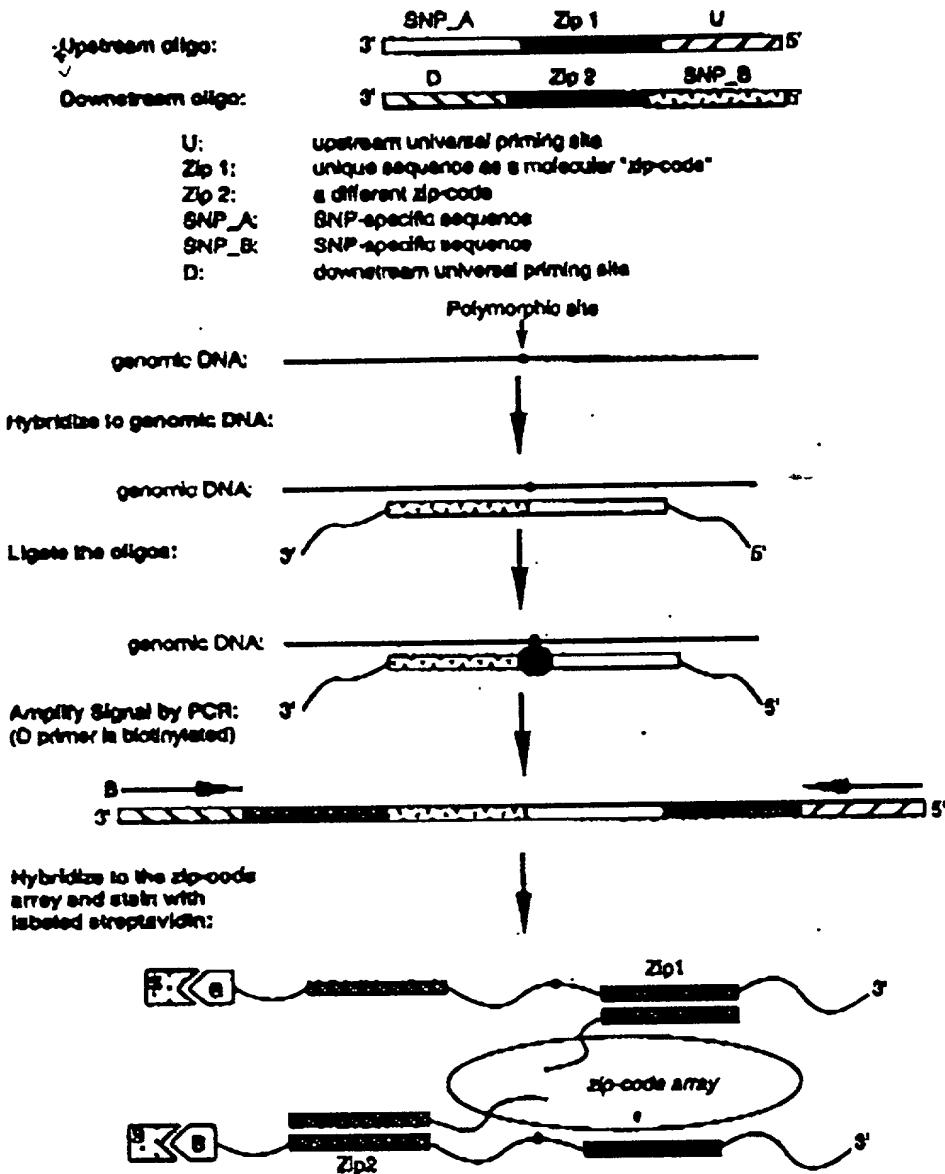
Figure 5

Whole-genome oligo-ligation strategy
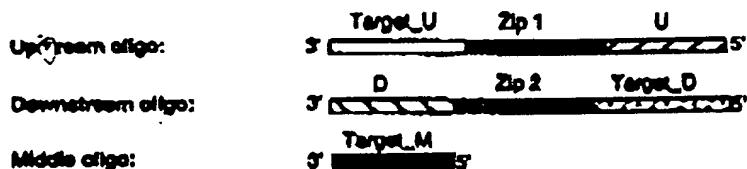
Upstream oligo:

Downstream oligo:

Middle oligo:

U:	upstream universal priming site
Zip 1:	unique sequence as a molecular "zip-code"
Zip 2:	a different zip-code
Target_U:	upstream target-specific sequence
Target_D:	downstream target-specific sequence
Target_M:	middle target-specific sequence
D:	downstream universal priming site

Target:

Hybridize to target:

Target:

Ligate the oligos:

Target:

Amplify signal by PCR:
(D primer is biotinylated)

Hybridize to the zip-code
array and stain with
labeled streptavidin:

Figure 6

unhybridize
probes.

removal/wash
of unhybridized
probes

denature

amplify
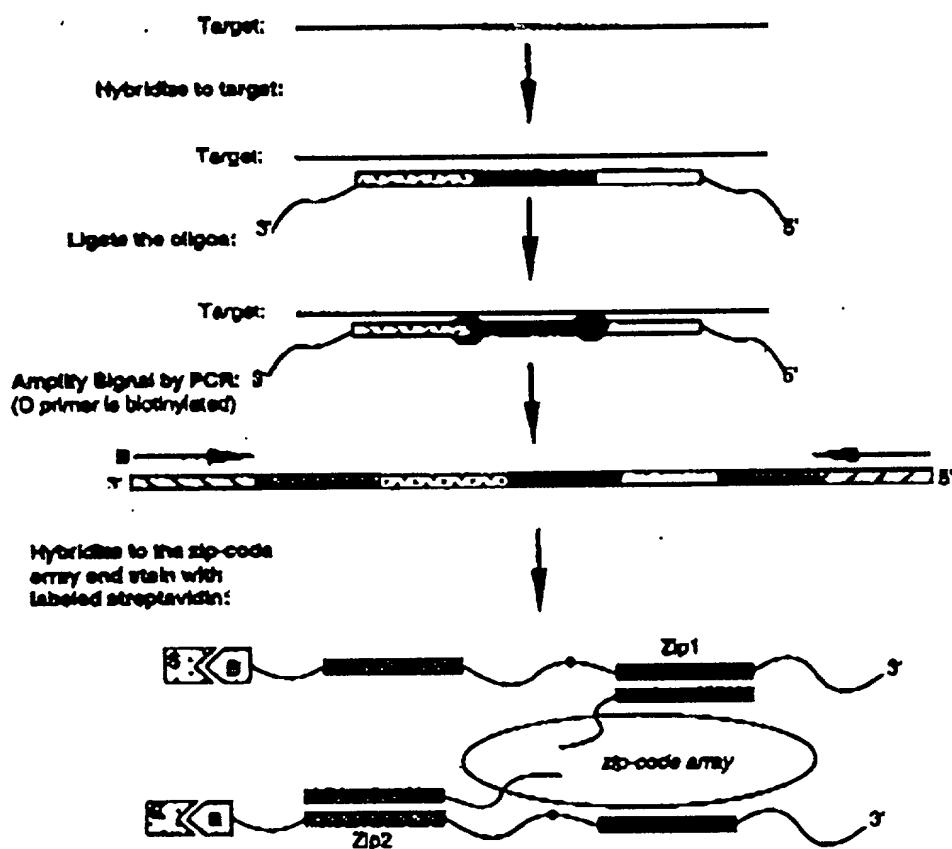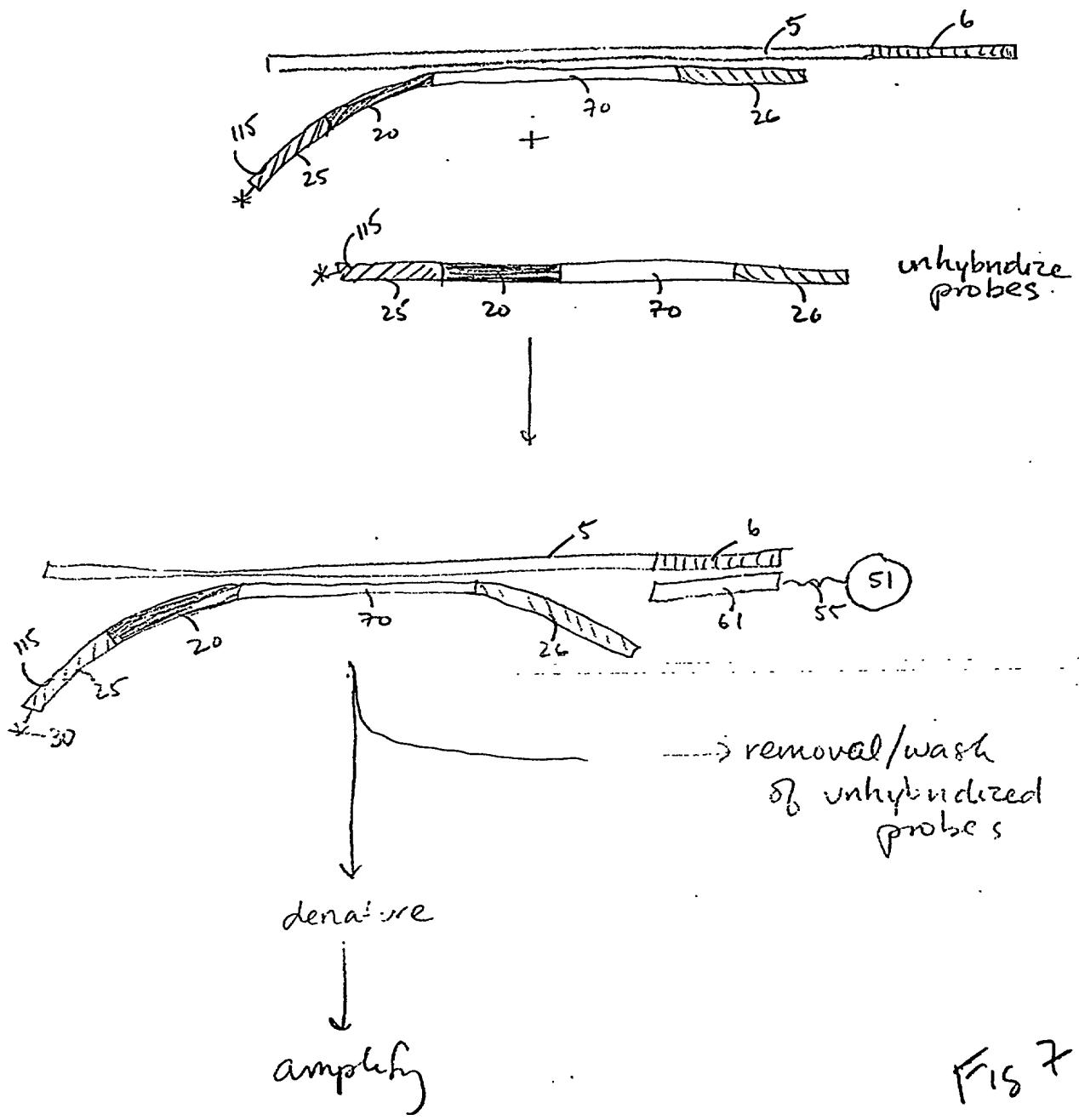
Fig 7
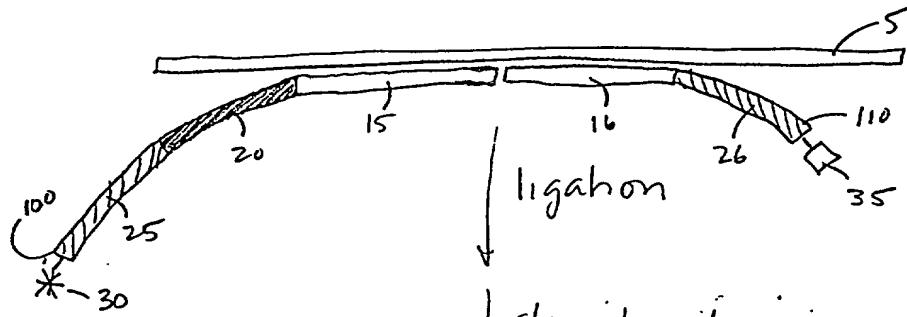
ligation

denaturation

addition
of exonuclease

+
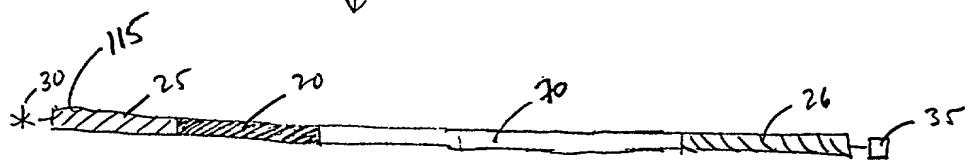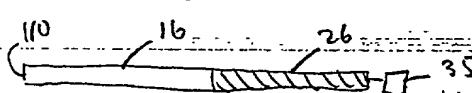
addition to
array,
wash away unbound

FIG 8

ligation

ligation

polymerase
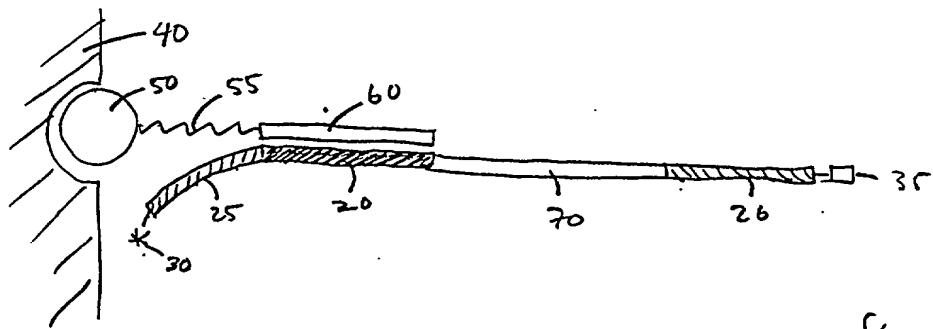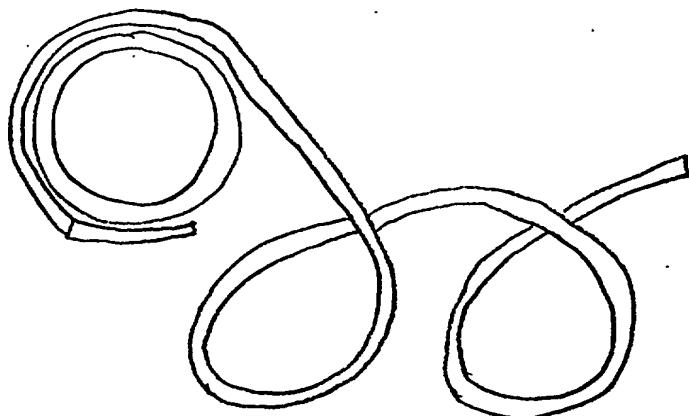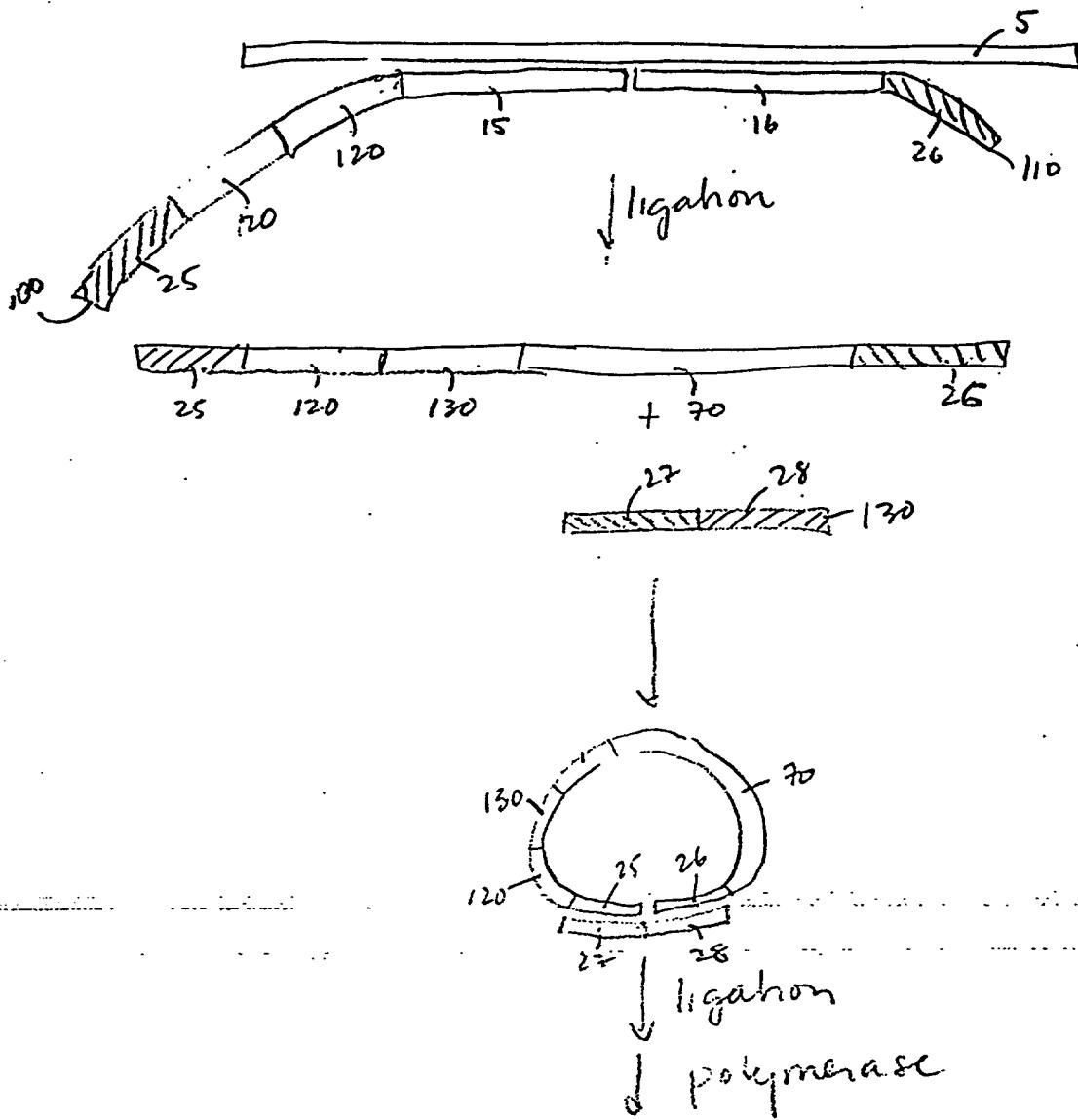
FIG 9
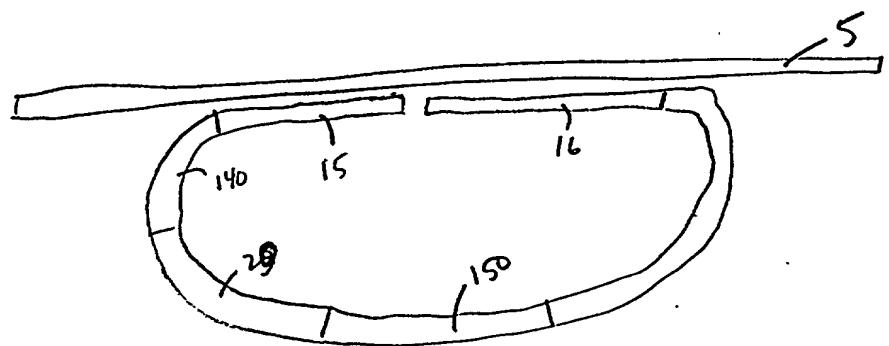
ligation, denaturation
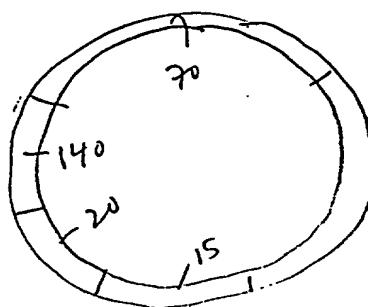
addition of
primer,
extension
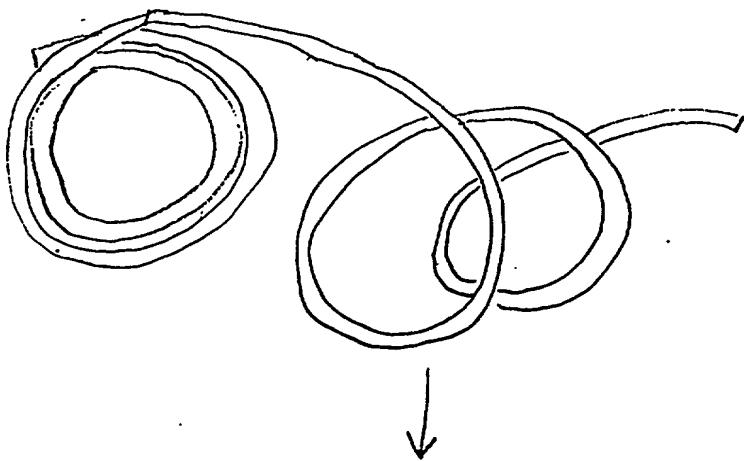
Fig 10

# Alternative Splicing Targets Selected for Microarray Analysis

1. GAPDH (constitutive splicing control, signal normalization).

2. FGFR2/KGF (mutually exclusive exons, internal cell type control):

KGFR (epithelial cells)

K   B

FGFR2 (fibroblasts)

3. Bcl-x (alternative 5′ ss):

Bcl-xL (inhibits PCD)

Bcl-xS (enhances PCD)
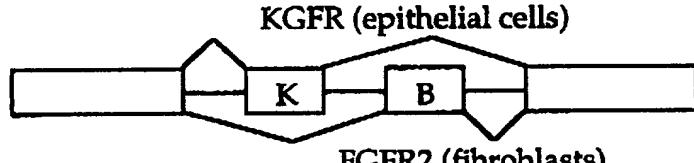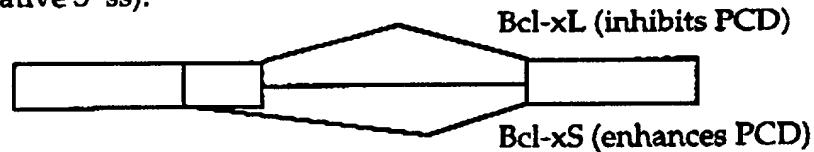
4. c-src (exon inclusion/exclusion):

N-src

N

C-src
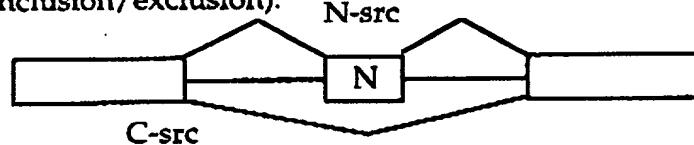
5. CASP2 (exon inclusion/exclusion):

Ich-IS (inhibits PCD)

Ich-IL (enhances PCD)

6. CASP9 (alternative 5′ ss):

9

9b (dominant negative form)
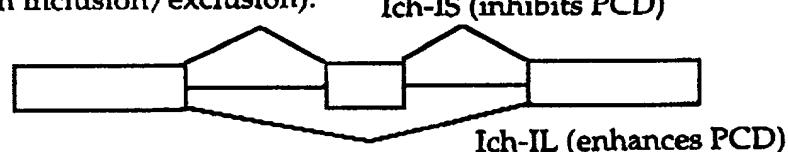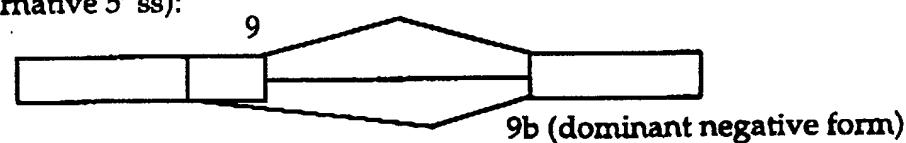
7. Fyn (src family tyrosine kinase, mutually exclusive exons):

brain-specific

7A   7B

thymus specific
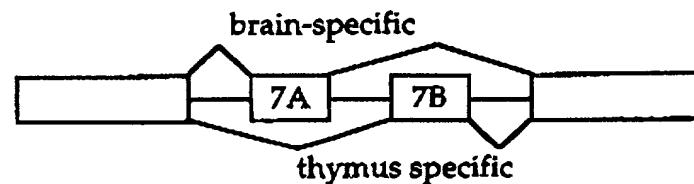
8. NOS1 (alternative promoters/alternative 5′ss):

FIG
11